Genetic risk factors in inflammatory abdominal aortic aneurysms: Polymorphic residue 70 in the HLA-DR B1 gene as a key genetic element

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Purpose: **Evidence of a genetic predisposition to the development of inflammatory abdominal aortic aneurysms (AAAs) exists as a positive family history in 17% of patients. Familial clustering and other similarities between inflammatory AAAs and giant cell arteritis (GCA), which possesses a genetic risk determinant mapped to the *HLA-DR* molecule, suggest a role of genetic risk factors in inflammatory AAAs. The purpose of this study was to explore whether patients with inflammatory AAAs express disease-relevant genes associated with the *HLA-DR* region on the short arm of chromosome 6.**

Methods: **Thirty-seven patients with histomorphologic findings of inflammatory AAA at operation were genotyped for the polymorphism of the *HLA-DR B1* and *HLA-DQ B1* alleles and compared to ethnically matched, healthy control subjects (n = 90).**

Results: **Distribution of *HLA-DR B1* alleles was nonrandom in patients with inflammatory AAAs versus control subjects. The *HLA-DR B1* alleles *B1*15* and *B1*0404* were enriched in patients with inflammatory AAAs compared with control subjects (47% versus 27%, and 14% versus 3%; $p < 0.05$, respectively). Analysis of functionally relevant amino acid polymorphisms encoded by the *HLA-DR B1* gene showed relevance at amino acid position 70. *HLA-DR B1* alleles overrepresented in patients with inflammatory AAAs express a glutamine substitution at position 70, whereas alleles disfavored in the patient cohort express a negatively charged aspartic acid. Distribution of *HLA-DQ B1* alleles were indistinguishable in patients and control subjects.**

Conclusion: **These data indicate that a genetic risk determinant can be mapped to the *HLA-DR B1* locus in patients with inflammatory AAAs. This association suggests a critical contribution of antigen binding in the pathogenesis of this disease. (J Vasc Surg 1997;25:356-64.)**

Inflammatory abdominal aortic aneurysms (AAAs) were originally described by Walker et al.[1] and represent 2% to 14% of all AAAs.[1-6] As we have recently reviewed, they are defined by the triad of thickened aneurysm wall, extensive perianeurysmal and retroperitoneal fibrosis and inflammation, and dense adhesions of adjacent abdominal organs (Fig. 1).[1-3] Patients frequently have the symptoms of fatigue, malaise associated with abdominal or back pain, and an elevated sedimentation rate.[6] The mean age of patients with inflammatory AAAs is 65 years, 5 to 10 years younger than patients with noninflammatory AAAs.[1-6]

We and others have shown indirect evidence for the role of genetic factors in the development of inflammatory AAAs.[2,7] A positive family history of aneurysms exists in 17% of patients, and clinical experience suggests clustering of these aneurysms in northern Europeans.[7] In addition, these aneurysms have a distinct tendency to occur in men.[2-7] These observations strongly suggest a role for genetic risk

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factors in their development; however, to date no direct evidence identifying a specific genetic risk determinant has been shown.

Although the cause of inflammatory AAAs is unknown, recent studies from Pearce et al.[12] and Tilson et al.,[41] as well as others, have provided insight into the pathogenesis of both inflammatory and noninflammatory AAAs.[8-11] These studies reveal several features that indicate an ongoing destructive immune response in the aortic wall.[8-11] Specifically, these studies and others have shown an immune process characterized by aortic wall–infiltrating macrophages, T lymphocytes (enriched in the $CD4^+$ population), and B lymphocytes.[10,12,13] Production of important cytokines and cellular adhesion molecules has been shown to be increased in aneurysmal aortic wall.[14-16] These cytokines and adhesion molecules lead to activation of proteolytic activity in the form of metalloproteinases,[8,9,17] which increase turnover of the matrix proteins, elastin, and collagen.[18-21] Subsequent loss of aortic wall integrity and tensile strength occurs as an aneurysm forms.[21] This inflammatory process appears to be accentuated in certain predisposed persons, and an inflammatory AAA develops at a relatively younger age. Also of interest, a high percentage of patients with inflammatory AAAs are current smokers, raising the question of the influence of tobacco on the pathogenesis of this disease.[7]

In searching for a unified hypothesis for the formation of inflammatory AAAs, we have been impressed with their similarity to another arterial disease, giant cell arteritis (GCA). Inflammatory AAAs share clinical and histopathologic similarities to GCA that, like aneurysms, show a tissue tropism for the medium and large arteries of the body.[22] Similar to patients with inflammatory aneurysms, patients with GCA frequently have symptoms of fatigue, fever, weight loss, and an elevated sedimentation rate, likely mediated by a systemic component of the inflammatory process.[22,23] Also common to these two arterial diseases is the clustering of disease among certain ethnic groups and or within families.

As in patients with inflammatory AAAs, studies in patients with GCA show that the large arteries are involved with tissue-infiltrating inflammatory cells, mainly T lymphocytes and macrophages, which center their immune process in the media of the arterial wall.[24-26] This inflammation in the arteries in GCA is associated with the destruction of the smooth muscle layer and fragmentation of the internal elastic lamina. Weyand et al.[26,27] and Wagner et al.[28] showed that the inflammatory process within the vessel wall is directed by $CD4^+$ helper T lymphocytes as well as by

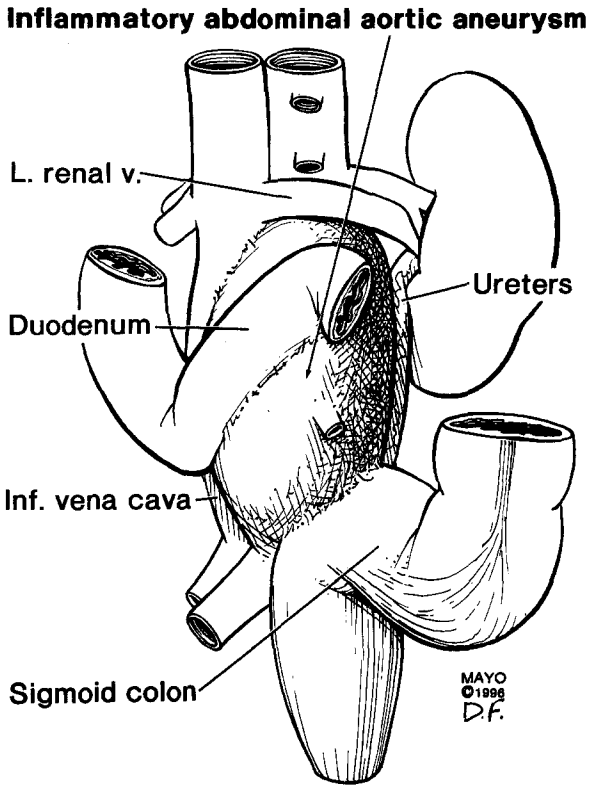

Fig. 1. Illustration of inflammatory abdominal aortic aneurysm (AAA) with adherent, adjacent abdominal organs.

macrophage activation. Molecular analysis of tissue infiltrating T cells has indicated that identical T cells proliferate at anatomically distinct sites of the inflammatory process. The most likely explanation for the activation of selected T cells in the tissue is the recognition of a tissue-residing antigen.[29]

Weyand et al.[30] showed direct evidence of a genetic risk determinant in the development of GCA that exists on the *HLA-DR* molecule within the class II major histocompatibility complex. The important location of this genetic determinant within the antigen binding groove of the *HLA-DR* molecule also suggests that antigen binding and presentation to the T lymphocyte is a critical event in the cause and pathogenesis of GCA. On the basis of the clinical and histopathologic similarities between GCA and inflammatory AAAs, we hypothesize that similar genetic risk determinants may exist that predispose persons to the development of inflammatory AAAs. Because of the distinct nature of these two diseases, we also hypothesize that the genetic risk determinant that exists for patients with inflammatory AAAs is distinct from and exists on a different location of the *HLA-DR* molecule than that for GCA. Characteriza-

Table I. Demographic and clinical characteristics of patients with inflammatory AAA

	Patients with inflammatory AAA (n = 37)
Age at operation (yr)	68
Sex (% male)	77
Smoker (% former or present)	89
Family history of aneurysms (%)	22
Peripheral vascular disease (%)	32
Coronary artery disease (%)	35
COPD (%)	40
Hypertension (%)	66
Elevated sedimentation rate (%) (16/23)	70
Aneurysm size (mean, cm)	6.8

AAA, Abdominal aortic aneurysm; *COPD,* chronic obstructive pulmonary disease.

tion of its location on the *HLA* molecule may allow for predictions on the nature of the immunogenic peptide bound in inflammatory AAAs and thus facilitate the search for disease-relevant antigens.

METHODS

Patients. Patients with classic recognizable inflammatory abdominal aortic aneurysms at the time of operation were entered into group 1 (n = 37). Patients in whom the diagnosis of inflammatory versus noninflammatory AAA was in question at the time of operation were excluded from the study population. No patients were excluded on the basis of race or sex.

Table I shows the demographics of the patient population (n = 37) with inflammatory AAAs. Ninety normal individuals were recruited as control subjects (group 2). All patients in group 1 and control subjects in group 2 were white. None of the control subjects used had any history of autoimmune disease. Persons with a family history of an inflammatory rheumatic disease were excluded.

DNA analysis. The *HLA-DR B1* alleles were determined by amplification of DNA extracted from peripheral blood mononuclear cells with primer sets specific for the allelic variants at the *HLA-DR B1* locus, followed by oligonucleotide hybridization to identify sequence variants amongst the allelic products. A polymerase chain reaction (PCR) with 30 cycles was used under the following conditions: denaturation for 30 seconds at 94° C, annealing for 30 seconds at 55° C, and extension for 30 seconds at 72° C. To identify sequence polymorphisms encoded by the second and third hypervariable region of the *HLA-DR B1* alleles, amplified products were blotted onto nitrocellulose membranes and hybridized with biotin-labeled oligonucleotide probes specific for sequence polymorphisms of the different *DR B1* alleles and subsequently developed with streptavidine–alkaline phosphatase reaction. The primer sets and the nucleotide probes used for the identification of the *HLA-DR B1* alleles have been described previously.[28,31]

HLA-DQ B1 alleles were determined by a nested PCR approach. DNA from peripheral mononuclear cells was amplified with a *DQ B1*–specific primer (TGCCCGCAGAGGATTTCGTG) and the *HLA-DQ B1* group–specific primers *(DQ B1*02/03/04,* TGCAAGGTCGTGCGGAGCT; *DQ B1*05/06,* ATCCCGCGGTACGCCACCTC) under the following conditions: denaturation for 30 seconds at 94° C, annealing for 30 seconds at 62° C, and extension for 1 minute at 72° C. The amplified products were adjusted, diluted, and reamplified with the *HLA-DQ B1*–specific primer and the appropriate *HLA-DQ B1*–allele-specific primers *(HLA-DQ B1*02,* GTCCACCGCCGCCCGTTT; *DQ B1*03,* CTCGCACACCGTGTCCAACTC; *DQ B1*05,* TCTGCACACCCTGTCCACCGA; *DQ B1*06,* TCTGCACACCGTGTGGAACTCG). To type for *HLA-DQ B1*04,* the appropriate PCR product was amplified with the *HLA-DQ B1*02/03/04* group–specific primer and an *HLA-DQ B1*04*–specific primer (ACCGAGCTCGTGCGGG). Each PCR included negative controls and DNA from lymphoblastoid cell lines from the Tenth International Histocompatibility Workshop as positive controls.

Statistical analysis. The frequencies of *HLA-DR B1* alleles in patients and in control subjects were compared by χ^2 test or Fisher's exact probability test as appropriate. Significance was defined a p value of less than 0.05.

RESULTS

Distribution of HLA-DR B1 alleles in patients with inflammatory AAAs. As shown in Fig. 2, the *HLA-DR B1* locus encodes for highly polymorphic molecules. The alleles *HLA-DR B1*01, 15, 03, 04, 11, 07,* and *13* are normally present in similar frequencies and are expressed by 20% to 25% of persons in a normal population. Alleles such as *HLA-DR B1*08, 09, 10,* and *14* are less frequent and are detected in 2% to 10% of all white donors. Conversely, patients with inflammatory AAAs display a nonrandom distribution of *HLA-DR B1* alleles.

The most frequent *HLA-DR B1* variant among the patients with inflammatory AAAs was *HLA-DR*

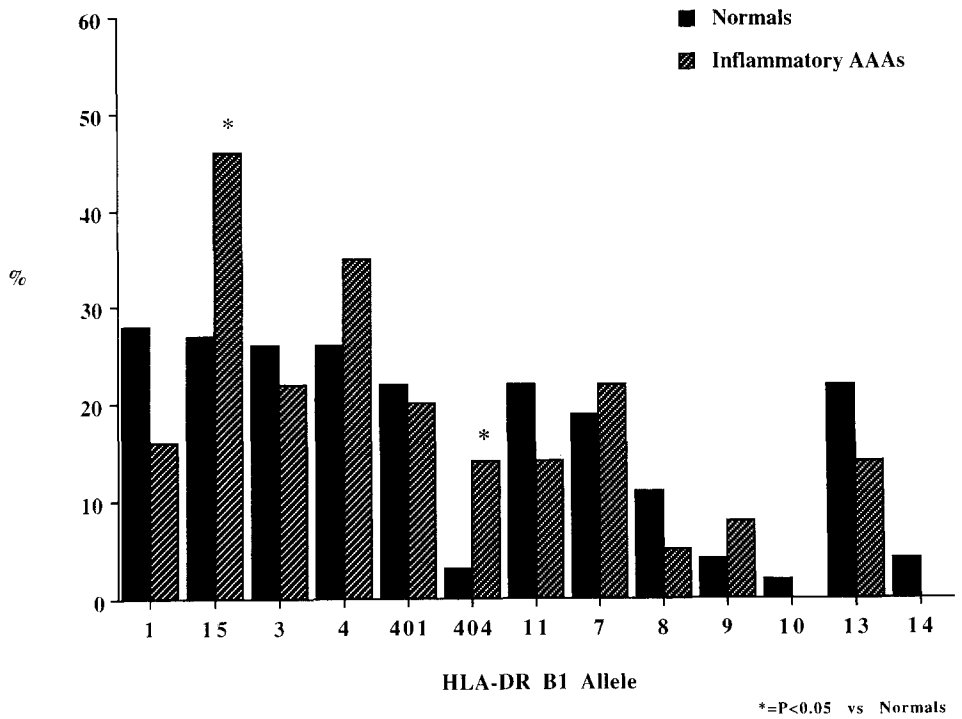

Fig. 2. Frequency of *HLA-DR B1* alleles in patients with Inflammatory AAAs versus control subjects.

*B1*15,* which was expressed in 46% of the patient cohort. This accumulation of *HLA-DR B1*15* donors in the patient population reached statistical significance ($p = 0.035$) compared with control subjects. In addition, a higher proportion of patients with inflammatory AAAs had inherited an *HLA-DR B1*04* allele, with 35% of patients typing positive for this allele. This trend did not reach statistical significance. The *HLA-DR B1*04* family includes a cluster of alleles that differ by minor sequence differences in the third hypervariable region of the gene. In the white population, *HLA-DR B1*0401* is the most frequent allelic variant, followed by *HLA-DR B1*0404, 0402,* and *0403.* Patients with inflammatory AAAs who are positive for *HLA-DR B1*04* transcribed an *0401* and *0404* allele in similar frequency (Fig. 2). This finding indicates that *HLA-DR B1*0404* individuals are enriched in the patient population. This enrichment reached statistical significance ($p = 0.032$) compared with control subjects. Thus patients in whom inflammatory AAAs develop are selected for the polymorphism of their *HLA-DR B1* gene, with an *increased* risk for *HLA-DR B1*15*$^+$ and *HLA-DR B1*0404*$^+$.

In patient cohorts with a biased distribution of *HLA-DR B1* genes, a compensatory *decrease* of non–disease-associated alleles can be expected. As shown in Fig. 2, a decrease in allele frequencies in patients with inflammatory AAAs also followed a nonrandom pattern. Patients with inflammatory AAAs have a decreased chance of typing *HLA-DR B1*13, 08, 11,* and *01.* The frequencies of *HLA-DR B1*03* and *07* were nearly indistinguishable between patients and control subjects. This pattern of *HLA-DR B1* alleles expressed among the patients suggests that the association of the disease with selected *HLA-DR B1* alleles does not simply reflect the increased disease risk of carriers of a single *HLA-DR B1* allele.

Nonrandom selection of position 70 in the HLA-DR B1 gene in inflammatory AAA patients. The major biologic function of *HLA-DR* molecules is to bind and present antigenic peptides for recognition by the T-cell receptor. The binding function is facilitated by accommodating polypeptide side chains in highly polymorphic binding pockets that are clustered in an antigen-binding cleft. The sequence polymorphisms characteristic for *HLA-DR B1* alleles transfer into polymorphic sites, shaping the antigen-binding pockets. One of the important binding pockets, pocket 4, has been mapped to include the amino acid positions 67, 70, 71, and 74. Pocket 4 extends from the floor of the antigen-binding groove to the wall formed by an α helix. As noted, within binding pocket 4, amino acid positions 70 is

	Amino Acid Position in Pocket 4			
	67	70	71	74
Favored Alleles				
B1*15	I	Q	A	A
B1*0404	L	Q	R	A
Disfavored Alleles				
B1*11	F/I	D	E/R	A
B1*08	I	D	R	A
B1*13	I	D	E/R	A
B1*01	L	Q	R	A

Fig. 3. Amino acid sequences of the favored and disfavored alleles in patients with inflammatory AAAs.

located in a critical position at the entrance of the pocket. In many *HLA-DR B1* alleles, position 70 is a negatively charged aspartic acid that favors interaction with positively charged peptide ligands. Alternatively, the *HLA-DR B1*–encoded β chain may express a noncharged glutamine in position 70. Because of differences in the charge of these two possible substitutions, the binding characteristics can change dramatically, depending on whether position 70 holds an aspartic acid or glutamine.

To examine the potential role of antigen-binding pockets in patients with inflammatory AAAs, *HLA-DR B1* alleles were analyzed for the sequence polymorphisms in positions 67, 70, 71, and 74. All of these positions are localized in the central region of *HLA-DR*–antigen interaction. As shown in Fig. 3, the two allelic variants enriched in the patient population shared a glutamine substitution (Q) in position 70. Conversely, three of the four alleles disfavored in the patient population expressed an aspartate substitution (D) in position 70. None of the other amino acids in polymorphic sites were informative in dissection of the two sets of alleles. The dichotomy that exists for position 70 in the inflammatory AAA group was not maintained for one of the alleles that occurred with decreased frequency in patients compared with control subjects, *HLA-DR B1*01*.

Allelic combinations at the HLA-DR B1 locus in patients with inflammatory aAAs. Whenever two different alleles are associated with a disease process, the question arises whether patients *who combine both* alleles have a higher risk of development of the disease. To approach this question, the genotype data were analyzed for patients who expressed both an *HLA-DR B1*15* allele and an *HLA-DR B1*0404* allele. Only one patient was heterozygous for *HLA-DR B1*15* and *0404*, indicating the lack of additive affects of both disease-associated haplotypes. In addition, no patients were typed as *HLA-DR B1*15* homozygous and one patient was genotyped as *HLA-DR B1*0404/0404*. Obviously, a single susceptibility allele is sufficient to confer risk.

Patients who lacked both *HLA-DR B1*15* and *B1*0404* frequently expressed *HLA-DR3* haplotype. More significantly, among the 10 patients who were negative for *HLA-DR B1*15* and *B1*0404*, three were homozygous for *HLA-DR B1*03*. Within the group of 90 white control subjects, only two persons were observed who were homozygous for *HLA-DR B1*03*.

Clinical characteristics of patients with enriched HLA-DR B1 alleles. The identification of two distinct alleles associated with the disease and the lack of additive effects of these two haplotypes suggests as one possibility that the patient cohort includes persons with two subtypes of disease. To explore this possibility, patients who type *HLA-DR B1*15*$^+$ or *HLA-DR B1*04*$^+$ were compared for clinical characteristics. The results of this comparison are given in Table II. Expression of the *HLA-DR B1*15* or *HLA-DR B1*0404* did not affect the age at diagnosis or sex. Although the use of tobacco was common in the patient population in general, it is intriguing to note that *100%* of the patients who typed positive for *HLA-DR B1*15* had a history of tobacco use. Furthermore, a higher percentage (60%) of patients smoked until the time of their diagnosis. Although statistical analysis of these observations of tobacco use in the *HLA-DR B1*15*$^+$ group did not reach significance compared with the *HLA-DR B1*04*$^+$ and *B1*15*$^-$*/B1*04*$^-$ groups, we believe that they represent interesting trends, possibly indicating the combination of genetic and environmental risk factors in the disease process. Also common in the *HLA-DR B1*15*$^+$ group was the presence of an elevated erythrocyte sedimentation rate in 90% of patients, suggesting a more severe or at least a systemic component of the disease. Of note, this is the same group that had the prominent history of tobacco use. The presence of an elevated erythrocyte sedimentation rate in the *HLA-DR B1*15*$^+$ group compared with the *HLA-DR B1*04*$^+$ group reached a p value of 0.04. Although statistical significance of some of these trends was lacking, the power of the analysis may have been limited by the number of patients in each of the subgroups; therefore these trends warrant further investigation with larger patient populations.

In contrast, a characteristic observed frequently

Table II. Clinical characteristics of patients with enriched HLA-DR B1 alleles

	*DR B1*15⁺ (n = 17)*	*DR B1*04⁺ (n = 10)*	*B1*15⁻B1*04⁻ (n = 10)*	*p Value*
Age (yr)	70	70	66	0.7
Sex (% male)	76	80	70	0.8
Smoker (% ever)	100	80	80	0.1
Smoker (% current)	60	40	40	0.5
Family history of aneurysms (%)	12	40	20	0.4
Elevated ESR (%)	90	33	71	0.04*
Aneurysm size (cm)	6	6.6	6.2	0.4

ESR, Erythrocyte sedimentation rate.
*$p < 0.05$ between 15^+ and 04^+.

in the *HLA-DR B1*04* group was that of a family history of aneurysms. Forty percent of patients with inflammatory AAAs who typed positive for *HLA-DR B1*04* reported a family history of aneurysms. This was nearly two times the rate of the population in general and more than three times the reported rate in the *HLA-DR B1*15⁺* group. These observations suggest allele-specific characteristics—that *HLA-DR B1*15⁺* patients have a lower rate of family history but require the use of tobacco to promote the disease—whereas the *HLA-DR B1*04* patients have a greater prevalence of family history and do not require the use of tobacco to promote the development of inflammatory AAAs.

Frequencies of the HLA-DQ B1 alleles in patients with inflammatory AAAs. *HLA-DR B1* genes are highly polymorphic but are inherited within a cluster of polymorphic genes in the *HLA-D* region located on the short arm of chromosome 6. The linkage disequilibrium within the *HLA-D* region raises the question of whether a particular locus or a locus in the near vicinity is the disease-associated element. For this reason, we extended our studies to the *HLA-DQ B1* gene. Allele-specific amplification with nested sets of primers was used to assign *HLA-DR QB1* genes to each of the 37 patients and 30 control subjects. The results are presented in Table III. The distribution of the *HLA-DQ B1* genes was essentially indistinguishable in both study cohorts. The association of inflammatory AAAs with allelic variants of the *HLA-DR B1* locus and the lack of an association with the *HLA-DQ B1* locus lend further support to the importance of *HLA-DR* molecules in the disease process.

Table III. Frequencies of HLA-DQ B1 alleles in patients with inflammatory AAA and in Normal subjects

HLA-DQ B1 allele	*Patients with inflammatory AAA (n = 37) %*	*Control subjects (n = 33) %*	*p Value*
*DQ B1*02*	46	30	NS
*DQ B1*03*	64	54	NS
*DQ B1*04*	3	12	NS
*DQ B1*05*	14	24	NS
*DQ B1*06*	46	45	NS

NS, Not significant.

DISCUSSION

Histocompatibility proteins are cell surface proteins that bind antigenic peptides intracellularly and present them at the cell surface for interaction with T lymphocytes[32,33] that, once activated, direct local inflammation by secretion of cytokines such as interleukin-2 and interferon-γ. This process is an important part of the immune response to foreign antigen.[32,33] Class II histocompatibility or *HLA* molecules are expressed by specialized antigen-presenting cells such as macrophages and dendritic cells.

Class II *HLA* molecules contain two different polypeptide chains, designated α and β, and are encoded by genes within the D region of the *HLA* complex located on chromosome 6.[34,35] In humans, this D region is divided into at least three subregions: DP, DQ, and DR. Each of these subregions contains at least one α gene and one β gene. The α and β chains come together as a heterodimer, which forms an antigen-binding cleft. The floor of the antigen-binding cleft is composed of eight antiparallel β-pleated sheets, whereas the side walls of the cleft are formed by α helixes.[35] Amino acid polymorphism within the β chain of the *HLA-DR* molecule and the α and β chains of the *HLA-DQ* molecule within the antigen-binding cleft mediates variations in the immune response of different individuals to different antigens and contributes to disease susceptibility.[36]

Within the antigen-binding cleft of the *HLA-DR* molecule are several small, highly polymorphic bind-

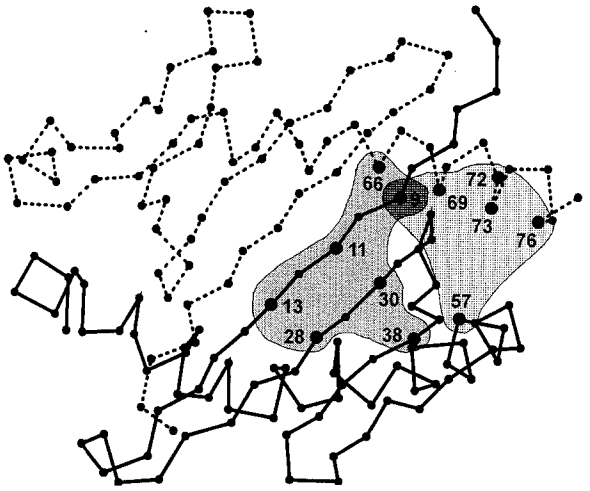

Fig. 4. Computer-generated model of the *HLA-DR* molecule with the giant cell arteritis (GCA) pocket highlighted. (Reproduced with permission from Stern LJ, Brown JH, Jardetsky TS, et al. Nature 1994;368:215-21. Copyright 1994 Macmillan Magazines Limited.)

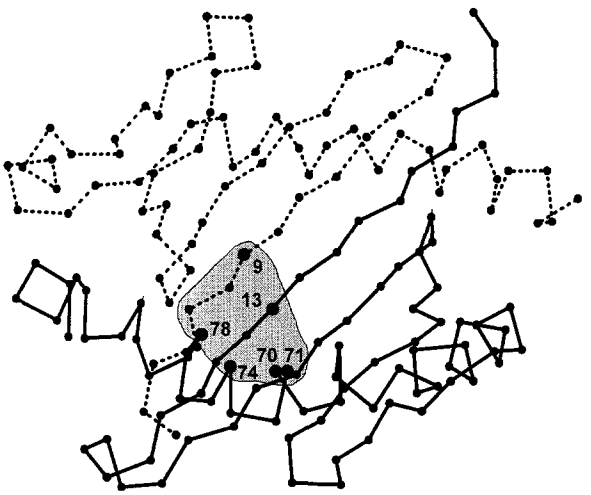

Fig. 5. Computer-generated model of the *HLA-DR* molecule with pocket 4 highlighted (Modified from Stern LJ, Brown JH, Jardetzky TS, et al. Nature 1994;368:215-21. Copyright 1994 Macmillan Magazines Limited. Used with permission.)

ing pockets. Specifically, five of these pockets appear to be important in accommodating side chains of antigenic peptides.[37] These polymorphic amino acid residues that line the side-chain binding pockets are responsible for the different peptide specificities of different *HLA-DR B1* alleles. Fig. 4 shows a computer model of the *HLA-DR* molecule, looking down into the antigen-binding cleft. The dotted line represents the nonpolymorphic α chain, and the solid line represents the polymorphic β chain. Highlighted is the amino acid sequence, which has been mapped as the genetic risk determinant in patients with GCA. The GCA pocket exists on the floor of the antigen-binding cleft of the *HLA-DR* molecule.

Because the inflammatory infiltrate in the arterial wall of patients with aneurysms suggests an ongoing immune response and because of the similarities between inflammatory AAAs and GCA, we explored in this study the *HLA* or immune response gene in patients with inflammatory AAAs. Our findings suggest an important contribution of amino acid position 70 of the *HLA-DR B1* chain, which is critically positioned at the entrance of binding pocket 4, extending from the floor of the antigen-binding cleft to one of the α helixes highlighted on the computer model in Fig. 5. This important location in the *HLA-DR* molecule accommodates side chains of antigenic peptides that interact with the *HLA-DR* molecule. Substitution of a Gln for a negatively charged Asp at the entrance to pocket 4 significantly changes the binding behavior of the pocket and therefore changes antigen selection.

Glutamine in a physiologic environment is most commonly a noncharged but polar amino acid. It can function as a hydrogen bond donor and may be found on the surface of or buried within a protein structure. Because of its relative compact nature, it is frequently found in α helixes. In contrast, aspartic acid is an acidic, negatively charged amino acid that is quite polar. Because of its charge, it is located more frequently on the surface of peptide structures. Aspartic acid can function as a hydrogen bond acceptor but is preferentially involved in protein-protein interactions by forming salt bridges. Enrichment of the expression of glutamine at position 70 therefore would change significantly the binding behavior of pocket 4 to side chains of antigenic peptides that interact with the *HLA-DR* molecule. Furthermore, this substitution at position 70 of pocket 4 likely changes its structural conformation and size. This finding in patients with inflammatory AAAs strongly suggests a critical role of antigen binding in the origin of this disease, and its distinct location on the *HLA-DR* molecule suggests disease specificity compared with GCA.

The cause of inflammatory AAAs remains an enigma. It appears that the cause is multifactorial: a combination of genetic, environmental, and endothelial factors that results in an antigen-driven, destructive inflammatory process in the arterial wall of

susceptible persons. Speculation regarding the antigen responsible for this process includes both endogenous and exogenous products. Breakdown products of elastin or red blood cells and oxidized low-density lipoproteins have been suggested as *endogenous* antigens.[38-40] A recent study by Tilson[41] that used immunoglobulin- and amino acid–sequencing techniques provides evidence that an *endogenous* autoantigen exists in aneurysmal disease that is similar to a microfibril-associated glycoprotein and raises the question of autoimmunity as an important mechanism in the pathogenesis of aneurysms.[41,42] Tanaka et al.[43,44] published evidence that the cytomegalovirus may play a role as an *exogenous* antigen in the pathogenesis of aortic diseases.

Regardless of the antigen, the observation of tobacco use among the *HLA-DR B1*15*$^{+}$ patients raises questions about its influence as a possible environmental promoter of the destructive inflammatory response resulting in inflammatory AAAs. This is a particularly appealing hypothesis, given that a high number of patients in the same group had elevated erythrocyte sedimentation rates. This evidence is observational in nature and does not offer mechanism; however, it is an pertinent observation that deserves further investigation both in larger clinical numbers of patients and in in vitro studies.

In summary, these findings support the studies of Drs. Pearce and Tilson and others who suggest that aneurysms may occur as an antigen driven inflammatory response within the arterial wall. In addition, these findings provide for the first time direct evidence of a genetic risk determinant to the development of inflammatory AAAs. Furthermore, they raise questions about the possible actions of tobacco use as an environmental promoter of this disease. Finally, this study extends the studies of Tanaka et al.[43,44] and others who provide possible antigenic instigators of the inflammatory response and, for the first time, allows characterization of actual antigen structure and charged composition. Studies such as this, characterizing the *HLA* molecule, may allow future predictions on the nature of antigenic epitopes of the selected immunogenic peptide bound and thus facilitate the search for disease-relevant antigens.

In conclusion, patients with inflammatory AAAs possess a genetic risk determinant that can be mapped to the *HLA* region. Specifically, patients with inflammatory AAAs preferentially express a neutral charge at the entrance of binding pocket 4 of the *HLA-DR* molecule. This localization of the genetic risk determinant distinguishes inflammatory AAAs from another *HLA-DR*–associated inflammatory disease that targets the wall of large arteries, GCA. Distinct polymorphic domains of the *HLA-DR* molecule are associated with inflammatory abdominal aortic aneurysms and giant cell arteritis, suggesting disease specificity. The role of different binding pockets in inflammatory abdominal aortic aneurysms and giant cell arteritis is compatible with a contribution of different antigens in disease pathogenesis. Further studies that combine clinical data with more extensive characterization of the *HLA* molecule may lead to the identification of disease-relevant antigens and environmental promoters of their pathogenesis.

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